Light absorption and growth response of *Dunaliella* under different light qualities

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Abstract

Dunaliella sp. (formerly *D. salina* MACC/C43) was batch-cultured under blue (BL), red (RL), or white light (WL) provided by light-emitting diodes (LED) and white light provided by fluorescent lamps (WF), respectively, to assess growth characteristic. Growth under blue light coupled with red light (BL + RL) was also measured and compared with that under BL, WL, and RL, respectively, in a laboratory-scale photobioreactor. Algae under monochromatic blue light had the highest specific growth rate (μ) with a value of 0.51 day^{-1}, followed by WF (0.49 day^{-1}), WL (0.47 day^{-1}), and RL (0.44 day^{-1}). Red light significantly enhanced Chl a and carotenoid contents, with values of 20.1 and 9.6 pg cell^{-1}, respectively. BL + RL increased the μ (0.20 day^{-1}) compared to those rates under pure BL or RL ranging from 0.17 to 0.18 day^{-1}, in batch culture for 14 days using a photobioreactor with 3.2 L working volume. Cells absorb more photons for photosynthesis in the blue band. Mean extinction coefficient calculated based on the modified Lambert–Bill law at the blue light region (430–480 nm) was 0.885 cm^{-1}, which is 22, 11, and 15% higher than that of the red light region (630–700 nm), green light region (500–560 nm), and photosynthetically active radiation region (400–700 nm), respectively. Blue light also improved the initial slope and the maximum O$_2$ production rate in a photosynthesis–irradiance curve, whereas it lowered the compensation irradiance and dark respiration rate. The photochemistry of photosystem II also displayed its optimal performance under BL. LEDs are a suitable light source for *Dunaliella* sp. growth, and BL + RL promotes rapid division of algal cells.

Keywords *Dunaliella* · Light quality · Blue light · Red light · Photosynthesis · Growth

Introduction

Dunaliella salina, a potential food supplement, accumulates β-carotene up to 10% of its dry weight (Ben-Amotz 1995; Borowitzka 1999; Chidambara Murthy et al. 2005; Lam and Lee 2014). Currently, all existing *Dunaliella* facilities,

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primarily located in Australia, Israel and China, adopt the outdoor open pond model, using high-salinity brine to produce algal biomass (Del Campo et al. 2007).

A two-stage cultivation process for *D. salina* β-carotene production has been studied (Ben-Amotz 1995). The alga is first grown in a suitable environment for the rapid growth and biomass accumulation and then the algae are transferred to stress conditions to enhance carotenogenesis, e.g., high irradiance, high salinity, high temperature, or N-deficient medium. This two-stage cultivation is possible in China, India, and Israel where the intensive raceway cultivation process is used. In stage one, a large number of algal cells are critical and necessary for the β-carotene production in the next step (Tafreshi and Shariati 2006; Lamers et al. 2010; Fu et al. 2013). However, in an open pond, where artificial regulation of temperature and light is unlikely, the density of algal cells rarely reached 1×10^6 cells mL^{-1} (Ben-Amotz 1995). In closed photobioreactors, cell density reach can up to 1×10^8 cells mL^{-1}, according to reported data (Kleinegris et al. 2011; Prieto et al. 2011; Hu and Sato 2017). This means that the number of algal cells in an open pond with 200 m^2 in area

(whether unmixed shallow open ponds in Australia or raceway-type in India, China and Israel) can be realized through a 600-L reactor. It is very attractive to cultivate *D. salina* cells in closed reactors for the later stage of carotenoid accumulation, since they offer high values of both photosynthetic efficiency and productivity (Prieto et al. 2011) providing steady and controlled conditions (Tredici and Zitelli 1997; Borowitzka 1999), especially in the context of land requirement.

Several studies have been conducted to explore the feasibility of culturing *Dunaliella* in closed photobioreactors (Kleinegris et al. 2011; Prieto et al. 2011). There is as yet no report of commercial production of this alga using a photobioreactor, neither a tubular reactor is successfully used for the production of *Haematococcus*, nor a column reactor suitable for most microalgae (Johnson et al. 2018). It is probable that closed photobioreactors will come to play an important role in the production of carotenoids, either for the growth of the algae or for the β-carotene accumulation stage of *Dunaliella* (Xu and Harvey 2019a, b). In photobioreactors, the intensity and the quality of the artificial light are two critical factors that must be considered by the designer, especially for the autotrophic growth of microalgae. Light provided by fluorescent lamps with various spectral qualities used to be the most popular lighting source (Blanken et al. 2013). Currently, light-emitting diodes (LEDs) have become one of the most important light sources. LEDs with different light spectra, e.g., red, blue, and white, are commercially available and can be used to illuminate microalgal cultures (Chen et al. 2011; Schulze et al. 2014; Radzun et al. 2015; Molina-Miras et al. 2018). Several studies have been performed to determine the growth of microalgae in response to various selected light regimes (Walter et al. 2011; Kula et al. 2014; Schulze et al. 2014; Vadiveloo et al. 2015). Published data show that the optimal spectra for microalgal growth are highly species-dependent, mainly attributed to the difference of light absorption and conversion efficiency due to inherent properties such as the pigment profile, cellular architecture, and chloroplast arrangement (Li et al. 2013; Moheimani and Parlevliet 2013; Glemser et al. 2016). For the algae of interest, investigating the optimal spectrum composition for their growth aids in the selection of a suitable light source.

To date, little attention has been paid to the effect of different monochromatic light qualities on the growth of this alga. Thus, the objective of this study was to investigate the influence of monochromatic light, e.g., red light, blue light, or the combined blue and red light emitted by LEDs, on the photosynthesis of *Dunaliella* at the green stage. The light absorption characteristic of this alga for different wavelengths was also measured. We predict that our results will facilitate the construction of more efficient closed photobioreactors, yielding greater biomass accumulation of *D. salina*. We have carried out further experiments in the reported reactors (Li et al. 2015)

to test the batch culture results and find the appropriate artificial light source.

Materials and methods

Microalgal strain and culture media

Dunaliella sp. MACC/C43 (formerly *D. salina* MACC/C43), from the algal culture collection at Ocean University of China (Qingdao, P.R. China) was inoculated into 250 mL Erlenmeyer flasks containing 125 mL of F/2 medium with salinity of 32 psu (Guillard and Rhyter 1962). It is worth mentioning that this strain can grow both at seawater salinity and 250 psu with optimum salinity ranging from 90 to 150 psu (Chen 2009). However, the carotenoid composition and a systematic examination of this strain are still lacking, and the previous name of this strain *D. salina* may be incorrect (Borowitzka and Siva 2007; Jang et al. 2009). Therefore, we adopt the name *Dunaliella* sp., instead of the original name, and provided the optimum salinity, aiming to avoid the potential confusion information. The cultures were maintained at 25 °C and 60 μmol photons m^{-2} s^{-1} provided by cool-white fluorescent lamps under a 14-h light/10-h dark cycle. The Erlenmeyer flasks were shaken manually four times per day to avoid sticking. Before the formal experiment, microalgae were cultured in LED white light under 80 μmol photons m^{-2} s^{-1} and continuous light for 5 days to exponential growth period. The algal cells at logarithmic growth stage were inoculated into new medium. This process was repeated for five times.

Light treatments in batch cultivation

To explore the effect of various light qualities, a batch culture was established under continuous illumination of 80 μmol photons m^{-2} s^{-1} in a Precision Constant Temperature Incubator (BPH-9272, Shanghai Yiheng Ltd., Shanghai, P.R. China) at 25 °C. The illumination was provided by four artificial light sources in different spectral regions, i.e., blue LED strips (for blue light, BL), red LED strips (for red light, RL), white LED strips (for white light, WL), and fluorescent lamps (for white light, WF), respectively (Conrad Electronics, Hirschau, Germany). Light spectra were measured with a spectroradiometer (PLA-20, Everfine's Quality Measurement Instruments, China) between 350 and 800 nm with 1-nm resolution (Fig. 1). LED light, i.e., BL, RL, and WL, was provided by the strips consisting of 80 diodes (5 rows of 16). The incident light hits the vessels from the same one side. The light intensity of each vessel is established by taking the mean of irradiance rate at three points along a vertical facade close to the vessel wall filled with water, measured using a portable light quantum meter (3415F type, pulse
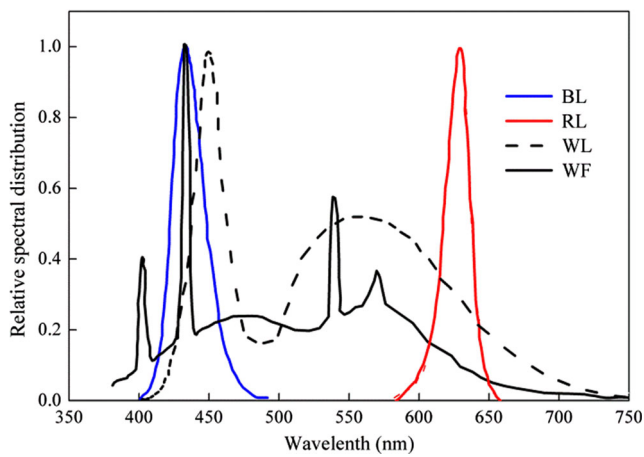
Fig. 1 Relative emission spectra of the light qualities used, blue light (BL), red light (RL), white LED light (WL), and white fluorescent light (WF), respectively

photoelectric sensor; Spectrum Technologies, Inc., USA). As for WF, fluorescent tubes arranged on the side of the container illuminated the container. The light intensity of each bottle is consistent with the LED group by adjusting the distance between the vessel and the tube. Five biological replicates were set for each light quality. To guarantee pure light quality, experiments under different light resources were carefully separated using compartments that were impervious to light.

Microalgal cultivation in a photobioreactor with different light qualities

A laboratory-scale flat-plate photobioreactor (PBR) with a working volume of 3.2 L (10 cm long, 8 cm wide, 50 cm high) was used for the cultivation (Li et al. 2015). The design parameters such as the ratio between downcomer and riser cross-sectional area and the ratio of height to diameter were 3:1 and 6:1, respectively. According to our previous report, the reactor used in this study has good mixing, with liquid circulation
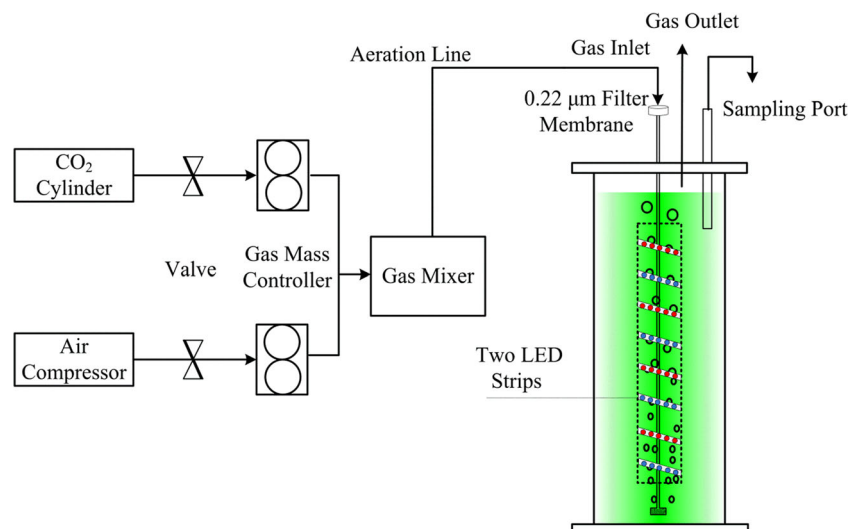
velocity 40.8 m s^{-1} and volumetric mass transfer coefficient 5.6×10^{-3} s^{-1}. Samples were obtained from a port 5 cm below the liquid level for further growth or pigment analysis. The aeration was kept constant using mass flow controllers at 0.3 L min^{-1} and was enriched with 2.5% CO_2. The algae were pre-acclimated to light intensity of 120 μmol photons m^{-2} s^{-1} for at least 20 days in LED white light as mentioned earlier. In order to investigate the effect of light quality on the growth of cells, all cultures were also maintained in batch growth mode. Growth was repeated three times for each light quality. Three biological replicates were collected every 2 days to measure the cell density. Light was provided via two inner set flexible LED strips on the transparent riser pipe of the PBR (Fig. 2). The growth of strain MACC/C43 under three light qualities, i.e., BL, RL, and RL + BL (with an intensity ratio of 1:1), was recorded at 120 μmol photons m^{-2} s^{-1}, respectively. LED light sources, i.e., WL, RL, and BL, are all surface-mounted light-emitting diodes. Each LED lamp bead emits light and different light quality lamp beads have a specific light intensity. The irradiance of each WL bead measured by using a 3415F type quantum meter is 1.0 μmol photons m^{-2} s^{-1}, and both RL and BL are 0.5 μmol photons m^{-2} s^{-1}. The incident light intensity of microalgal culture under different light qualities was set by controlling the number of light beads for each specific color. We lit 120 white beads, 240 red beads, and 240 blue beads, respectively, to create 120 μmol photons m^{-2} s^{-1} of white, red, and blue light. Similarly, 120 red and 120 blue beads were simultaneously lit to provide BL + RL (Li et al. 2019).

Measurement of growth

The specific growth rate in the exponential growth phase (μ) was calculated based on the following equation (Moheimani et al. 2013):

Fig. 2 Schematic diagram of the experimental setup and instrumentation

$$\mu = (\ln N_2 - \ln N_1)/(T_2 - T_1)$$

where N_2 (final) and N_1 (initial) are cell densities (cells mL^{-1}) determined using a hemocytometer at times T_2 and T_1, respectively.

Quantification of the photosynthetic pigments

Ethanol (99.5%) was used for the extraction of chlorophyll and carotenoids. Chlorophyll a (Chl a), Chl b, and total carotenoids (Car) were extracted and quantified as described by Ritchie (2008) and Jensen (1978) using a dual-beam UV–visible spectrophotometer (Hitachi, U-2900 UV/VIS, Japan) with quartz cell as follows:

$$\text{Chl a} = 0.0604 \times A_{632\,nm} - 4.5224 \times A_{649\,nm} + 13.2969$$
$$\times A_{665\,nm} - 1.7453 \times A_{696\,nm}$$

$$\text{Chl b} = -4.1982 \times A_{632\,nm} + 25.7205 \times A_{649\,nm} - 7.4096$$
$$\times A_{665\,nm} - 2.7418 \times A_{696\,nm}$$

$$\text{Car} = (1000 \times A_{470\,nm} - 2.05 \times \text{Chl a})/245$$

where A represents the absorbance at the indicated wavelength. Algal cells on the fifth day were sampled to determine the pigment content.

Photosynthesis–irradiance (P–E) curve of microalga under different light qualities

Oxygen evolution and uptake rates were determined in an iodine number flask (100 mL, total volume) filled with an algal suspension having a cell density of about 1.0×10^5 cells mL^{-1}. First, the microalgae cultured in LED white light as described in the "Microalgal strain and culture media" section were precultured under each light quality for 2 h. Second, the biomass was measured as described by Zhu and Lee (1997). The dissolved oxygen concentration (DO) in the alga solution was then measured using a polarographic sensor with a DO meter (Models 58 and 5100; YSI Inc., USA) every 10 min for 30 min. The net photosynthetic rate (P_{net}) was expressed by the rate of variation of the dissolved oxygen concentration with time. After that, the DO concentration was recorded every 10 min for 30 min in the dark. The slope of a linear regression was determined from the data of the second 30-min measurements to estimate the respiratory rates (R_d). Photosynthetic rates were determined at 40, 60, 80, 140, 180, 220, 280, 320, and 460 μmol photons m^{-2} s^{-1} ($N = 3$ replicates/level) at 25 °C. Both the photosynthetic evolution and respiratory O$_2$ uptake rate are expressed as the oxygen release rate per unit dry weight of biomass (μmol O$_2$ g^{-1} h^{-1}).

The P–E curve was fitted using an exponential equation (Jassby and Platt 1976):

$$P_{Gross} = P_m \times \tanh(\alpha \times E/P_m)$$

where P_{Gross} is the gross O$_2$ production rate, which is the sum of P_{net} and R_d. P_m is the maximum O$_2$ production rate and α is the initial slope. Saturation irradiance (E_k) and compensation irradiance (E_c) were calculated as P_m/α and R_d/α, respectively. The fitting was performed using the software origin 8.0 (OriginLab, USA). Curve fit was calculated with the Nonlinear Curve Fit function of this software using the least squares method comparing differences between measured and calculated data (Houlbrèque et al. 2004).

Light absorption of algal cells and chlorophyll fluorescence measurements

The average extinction coefficients at different wavebands, i.e., BL (400–480 nm), green light (GL, 500–560 nm), RL (600–650 nm), and photosynthetic active radiation (PAR, 400–700 nm), were determined as described by Li and Meng (2014). Microalgae precultured in LED white light as described in the "Microalgal strain and culture media" section were used to determine the light absorption in order to avoid the possible influence of light acclimation on light absorption. The transmittances were recorded by scanning the supernatant. The average light transmittance (T) and mean extinction coefficient (a) of algal suspension was calculated with the equation below.

$$T = \frac{\sum_{\lambda=\lambda_d}^{\lambda_u} S(\lambda) \times T(\lambda) \times V(\lambda)}{\sum_{\lambda=\lambda_d}^{\lambda_u} S(\lambda) \times V(\lambda)}$$

$$\text{Ln}(T) = 2 \times \text{Ln}(1-r) - a \times L$$

where λ_d and λ_u are the lower-limit wavelength and the upper-limit wavelength for each light quality, respectively. $T(\lambda)$ represents the monochromatic light transmittance of each wavelength (%). $S(\lambda)$ is the relative spectral power distribution on the corresponding wavelength (W). $V(\lambda)$ is the efficiency of spectral illumination of the corresponding wavelength. The values of $S(\lambda)$ and $V(\lambda)$ can be obtained from the information given by the International Commission on Illumination (CIE) and the National Standardization Technical Committee (2008). According to the CIE, the sampling interval to obtain values of $S(\lambda)$ and $V(\lambda)$ is 5 nm in the range 400–700 nm. The specific extinction coefficient of mixed light quality was expressed by the average value of each monochrome light quality.

The chlorophyll fluorescence of photosystem II (PSII) was measured using an FMS-2 pulse-amplitude modulated fluorometer (Hansatech Instruments, UK) as described by Zhang et al. (2015). Algal cells cultivated in BL, RL, WL, and FL, respectively, for 5 days in batch cultivation were sampled to determine the chlorophyll fluorescence. All measurements

were performed with microalgae that had been dark-adapted for 20 min at 25 °C.

Statistical analysis

Data in the figures represent the averages of replicates ± standard error of the mean (SEM) and were subjected to one-way ANOVA and Tukey tests performed using SPSS 17.0 software (SPSS Inc., USA). Differences were considered significant at a probability level of $P < 0.05$.

Results

Batch cultivation of *Dunaliella* sp. under four light qualities

During the cultivation period of 6 days, strain MACC/C43 exhibited exponential growth under all four light qualities, and the highest cell density was observed in the BL treatment (Fig. 3a). The maximum specific growth rate μ (0.51 ± 0.008 day^{-1}) was also observed under the BL conditions, followed by WF (0.49 ± 0.001 day^{-1}), WL (0.47 ± 0.002 day^{-1}), and RL (0.44 ± 0.014 day^{-1}) (Fig. 3b). RL significantly increased the pigment content, both in the unit volume of algal suspension and in the single cell (Fig. 3c, d). All pigments, i.e., Chl *a*, Chl *b*, and total carotenoids (Car)

displayed no significant difference between the BL and WF treatments.

P–E curve parameters of *Dunaliella* sp. under different light qualities

Values of the gross photosynthetic rate increased steeply with increased light intensity under relatively low light conditions, and then basically remained constant under higher light conditions (Fig. 4). The parameters under the four light qualities were determined to be 1.99–2.52 μmol O$_2$ g^{-1} h^{-1} for the P_m, 0.016–0.023 μmol O$_2$ g^{-1} h^{-1} for α, 0.55–1.68 μmol O$_2$ g^{-1} h^{-1} (μmol photons m^{-2} s^{-1})$^{-1}$ for the R_d of the model, and 108.2–122.3 μmol photons m^{-2} s^{-1} for E_k. From these parameters, the E_c and the maximum P_{net} were calculated to be 23.6–103.4 μmol photons m^{-2} s^{-1} and 0.31–1.97 μmol O$_2$ g^{-1} h^{-1}, respectively. The P_m and the maximum P_{net} were both the highest in BL (2.52 and 1.97 μmol O$_2$ g^{-1} h^{-1}, respectively) and the lowest in RL (1.99 and 0.31 μmol O$_2$ g^{-1} h^{-1}, respectively). In contrast, α, E_k, and E_c were the lowest under BL and the highest under RL (Table 1).

Light absorption of *Dunaliella* sp. in different spectral regions

Light is absorbed by *D. salina* predominantly through Chl a, Chl b, and Car (Fig. 5a). The mean extinction coefficient in the

Fig. 3 The growth curve (**a**), maximum specific growth rate (**b**), pigment content per cell (**c**), and volumetric pigment concentration (**d**) of *Dunaliella* sp. MACC/C43 batch-cultivated under blue light (BL), red light (RL), white LED light (WL), and white fluorescent light (WF), respectively. Values are the averages of five biological replicates ± SEM. Car represents total carotenoids. Data of each parameter with different letters (i.e., a, b, c, and d in graph **b** and a, b, c, ab and bc in graph **c** and **d**) are significantly different in each index group ($P < 0.05$)
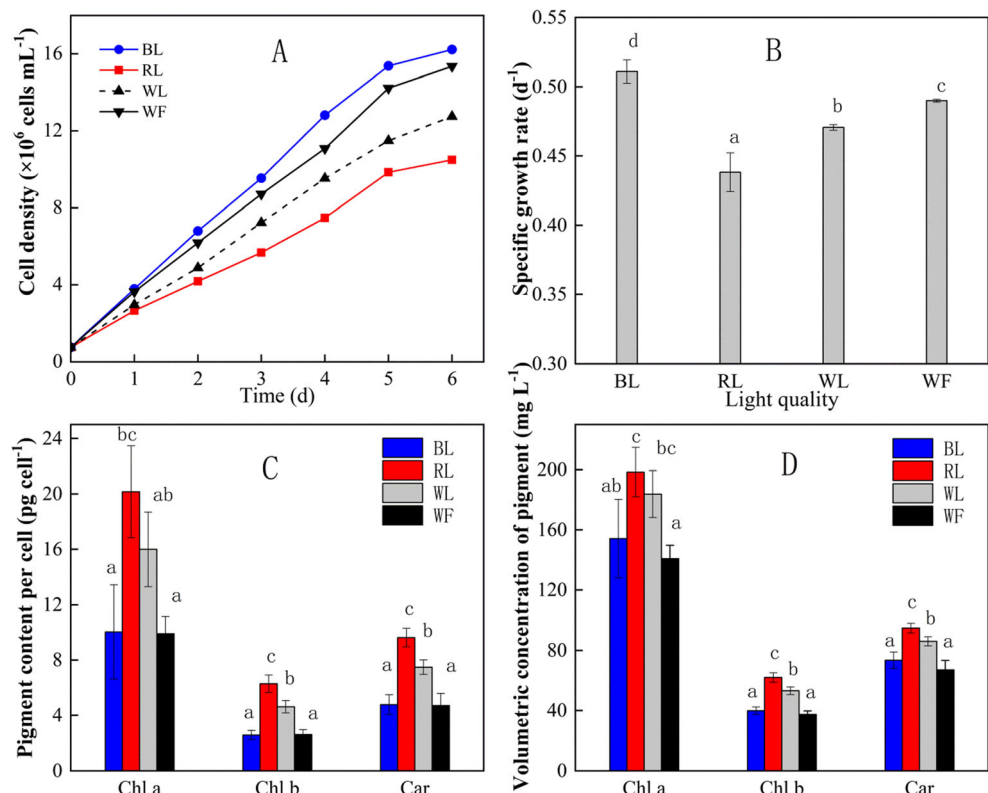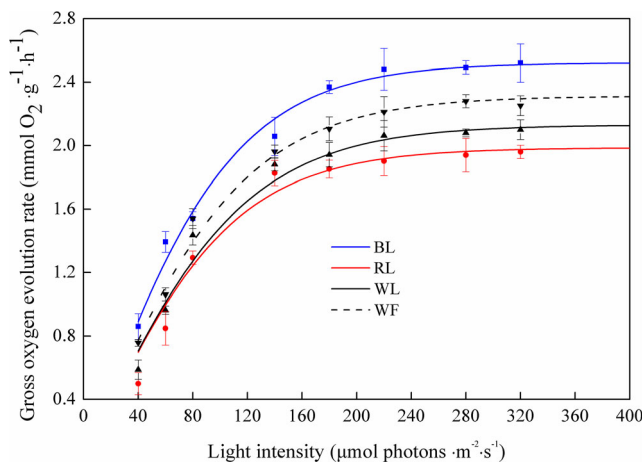
Fig. 4 Light response curve for the gross photosynthetic rate of *Dunaliella* sp. MACC/C43 cells under blue light (BL), red light (RL), white LED light (WL), and white fluorescent light (WF), respectively. Values are the averages of five biological replicates ± SEM

BL region was higher than those of the RL, GL, and PAR regions (Fig. 5b).

Effect of light quality on PSII photochemistry

Data in Fig. 6 shows that (i) the maximum fluorescence yield (F_m) under RL and WL was significantly higher than those of BL and WF; (ii) the maximum quantum yield of PSII photochemistry (F_v/F_m) maintained the highest value under BL, followed by WF, RL, and WL; (iii) the effective quantum yield of PSII (Φ_{PSII}) under both BL and WF was slightly higher than that of RL and WL, although no significant difference was observed between all the treatments; (iv) the photochemical quenching coefficient (qP) showed a pattern similar to that of F_v/F_m, with the highest values found under BL and WF; (v) nonphotochemical quenching (NPQ) under RL and WF light conditions was markedly higher than that under WL, and BL exposure gave the lowest level of all.

Table 1 The maximum gross photosynthetic O_2 evolution rate (P_m), respiration rate (R_d), light-harvesting and photosynthetic conversion efficiency (α), saturation irradiance (E_k), and compensation irradiance (E_c) of *Dunaliella* sp. MACC/C43 under blue light (BL), red light (RL), white LED light (WL), and white fluorescent light (WF)

Parameter	Light quality			
	BL	RL	WL	WF
P_m	2.52 ± 0.02	1.99 ± 0.04	2.13 ± 0.02	2.31 ± 0.03
α	0.023 ± 0.001	0.016 ± 0.001	0.018 ± 0.001	0.020 ± 0.001
R_d	0.55 ± 0.02	1.68 ± 0.04	1.46 ± 0.03	0.73 ± 0.02
E_k	108.2 ± 4.00	122.3 ± 5.47	116.2 ± 5.66	115.1 ± 4.30
E_c	23.6 ± 0.25	103.4 ± 4.22	79.6 ± 3.00	36.3 ± 0.84
R^2	0.9896	0.9950	0.9952	0.9960

## Growth response of *Dunaliella* sp. to light quality in PBR

To further optimize the artificial light source, strain MACC/C43 was cultivated in a PBR with four LED light qualities at the same intensity (120 μmol photons m^{-2} s^{-1}). As demonstrated in Fig. 7, for growth of algal cells under BL, BL + L, WL, and RL, cell populations increased in all the treatments, with the highest value (4.00×10^7 cells mL^{-1}) observed under combined BL + RL light conditions. For monochromatic light, BL was more suitable for algal growth than RL, which was consistent with the results for batch cultivation in flasks (Fig. 3a). The growth of the algae under WL was slightly faster than that under RL, but significantly lower than those of BL and BL + RL.

Discussion

In the design and operation of a microalgal cultivation system, the use of an appropriate light source is a key factor that requires consideration. The optimal wavelength varies from species to species. For example, *Nannochloropsis* sp. and *Haematococcus pluvialis* display the highest biomass yield under red light (Katsuda et al. 2004; Vadiveloo et al. 2017). However, blue light favors the growth and photosynthetic characteristics of *D. salina*, *Isochrysis galbana*, *Chaetoceros gracilis*, and *Heterocapsa circularisquama* (Gorai et al. 2014) and astaxanthin production in *H. pluvialis* (Katsuda et al. 2006). Our results also indicate that light quality significantly regulates the growth of *Dunaliella*, in both batch and closed PBR cultivation.

BL was a promising monochromatic illumination for cell growth in the indoor cultivation of strain MACC/C43, while illumination with RL increased the level of both Chl a and the total carotenoids (Fig. 3). Helena et al. (2016) reported that the growth of *D. salina* was regulated by LED quality, and the maximum cell densities under BL using the Walne medium and the ZA medium were both higher than those that were illuminated with RL. The current study further indicated that the highest specific growth rate of strain MACC/C43 was obtained using RL + BL together in a laboratory-scale PBR (Fig. 4). Wagner et al. (2016) found that the pigment concentration (Chl *a*, Chl *b*, and Car) of *Chlamydomonas reinhardtii* exposed to a dichromatic illumination of red and blue light at 25 μmol photons m^{-2} s^{-1} decreased with the decrease of red light proportion (from 80 to 50%). We can speculate that the enhanced growth of strain MACC/C43 exposed to combined LED light with blue and red wavelengths could be attributed primarily to the high growth rate under BL and higher pigment synthesis under RL. Similarly, Fu et al. (2013) found that combining RL (75%, peak at 470 nm) with BL (25%, peak at 660 nm) with a total incident photon flux of
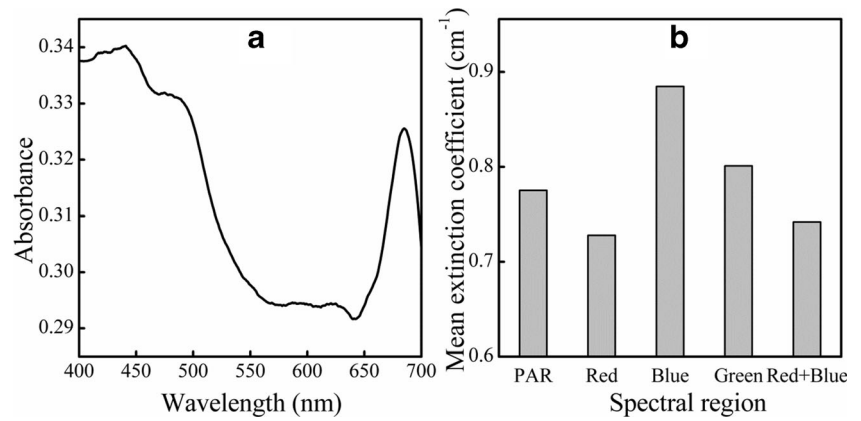
170 μmol photons m^{-2} s^{-1} resulted in a higher growth rate of *D. salina* compared to the values under RL at the same irradiance.

In green algae, the antenna pigment molecules (e.g., Car and chlorophyll) absorb light in different spectral regions

Fig. 6 The effect of light quality on the maximal fluorescence yield of the dark-adapted state (F_m), maximal quantum yield of PSII photochemistry (F_v/F_m), the effective quantum yield of PSII (Φ_{PSII}), photochemical quenching coefficient (qP), and nonphotochemical quenching (NPQ) of *Dunaliella* sp. MACC/C43. Values are the averages of six biological replicates ± SEM. Data with different letters (i.e., a, b, and c) are significantly different ($P < 0.05$). BL, RL, WL, and WF represent blue light, red light, white LED light and white fluorescent light, respectively

(chlorophyll $\lambda = 450–475$ nm and $\lambda = 630–675$ nm and Car $\lambda = 400–550$ nm) (Jahns and Holzwarth 2012). Chl a acts as a reaction center in the photosystems, though it is also a part of the antenna complex. Carotenoids primarily harvest blue light and transfer energy to the photosystem reaction centers (Gorai et al. 2014; Nisar et al. 2015). When light energy passes through the culture medium and reaches a microalgal cell, the light absorption efficiency of the cell and the spectral distribution of the incident light determine the available light energy for photosynthesis (Dubinsky et al. 1986). In the cells, pigments are contained in chloroplasts within an intricate molecular environment. The constituents of the cells such as proteins, lipids, pigments, and carbohydrates have their own optical properties that reduce the energy of photons reaching the PSII reaction center (Johnsen and Sakshaug 2007). The ratio of light energy absorbed by pigments to that absorbed by *Dunaliella* cells is unclear, and it is an interesting subject that needs to be investigated further. In this study, the higher growth rate under BL compared to that under WF or WL suggests that more blue photons were absorbed by the cells, which might play a dominant role in driving photosynthesis, although the same quantum flux density was supplied at the blue, red, green, and PAR ranges (Fig. 6). The photosynthetic activity of *Dunaliella* sp. MACC/C43 exposed to blue light was also enhanced (Table 1, Fig. 6). The highest P_m was observed in BL grown cells, as already reported for some other marine microalgae (Marchetti et al. 2013; Gorai et al. 2014), indicating that the cells expressed higher photosynthetic activity at saturating light intensities than when grown under RL, WL, and WF. The increase in activity can be attributed to an enhanced electron transfer supported by a higher F_v/F_m or qP under BL, as shown in Fig. 7. It is worth noting that our results did not exclude the possibility that blue light enhanced the activity of ribulose-1,5-diphosphate carboxylase/ oxygenase (Rubisco), thereby accelerating algal growth. It has been found that blue light increased the synthesis of Rubisco in higher plants and microorganisms, including green algae (Ruyters et al. 1984; Roscher and Zetsche 1986; Eskins et al. 2010). The effective band ranged from 430 to 510 nm,
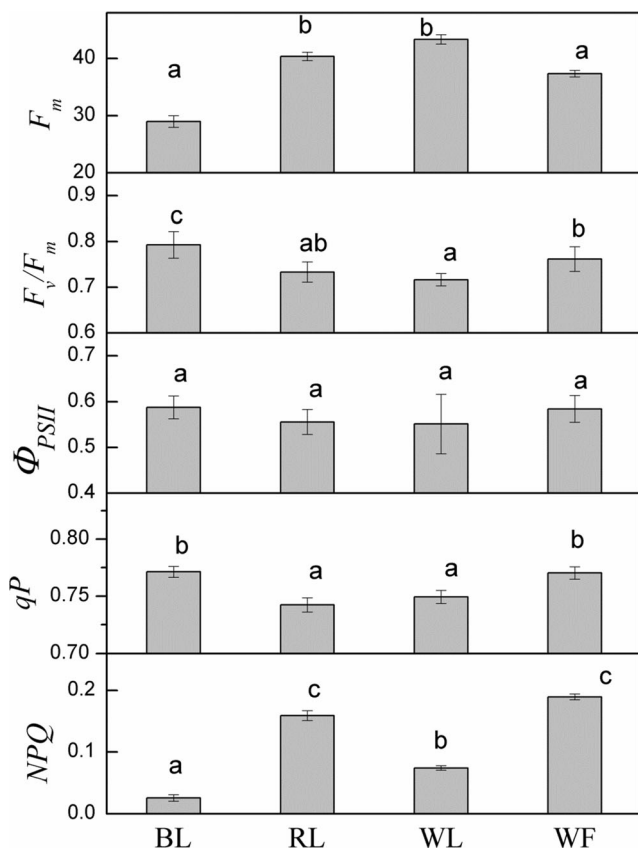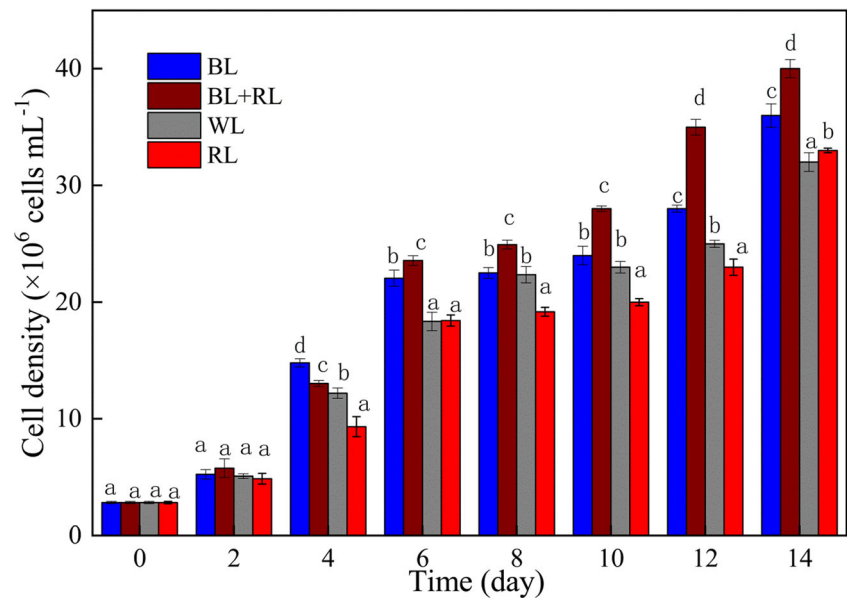
Fig. 7 Cell density of *Dunaliella* sp. MACC/C43 in a flat-plate photobioreactor for four light qualities, respectively. BL, RL, and WL represent blue light, red light, and white LED light, respectively. BL + RL represents a mixture of BL and RL at 1:1 light intensity. Data with different letters (i.e., a, b, c, and d) are significantly different ($P < 0.05$)

and the maximal effect was at 460 nm. The BL adopted in our study has a spectrum of 400 to 500 nm with a peak of 450 nm (Fig. 1). This question merits further investigation in the future.

Moreover, previous studies have also reported that blue light increased the α in some green or golden microalgae, such as *Scenedesmus obliquus* (Brinkmann and Senger 1978), *Prorocentrum mariae lebouriae* (Vogel and Sager 1985), *Dunaliella tertiolecta* and *Cyclotella nana* (Wallen and Geen 1971), and *Isochrysis* sp. (Marchetti et al. 2013). Our finding was consistent with these results. In the model, α is the initial slope of line at low PAR values created by relating P_{Gross} to E, which provides a measure of quantum efficiency (Schreiber 2004). Higher quantum efficiency under BL was also supported by the improved F_v/F_m coupled with decreased F_m (Fig. 7) and pigment contents (Fig. 3). E_k is a measure of the point where the light saturation dominates, also known as the minimum saturation level. This parameter was maintained almost unchanged under four light qualities, with the values ranging from 108.2 to 122.3 μmol photons m^{-2} s^{-1} (Table 1). Usually, light intensity higher than E_k causes photoinhibition in plants and, thus, results in the decrease of F_v/F_m (Marchetti et al. 2013). The intensity of incident light in our study (80 μmol photons m^{-2} s^{-1}) was not high enough to induce photoinhibition in the algal cells. Variations in the growth of the cells under different light conditions were primarily caused by light quality. In the literature, the R_d of some microalgae, e.g., *S. obliquus* (Brinkmann and Senger 1978), *Rhodomonas salina* (Hammer et al. 2002), and *D. tertiolecta* (Rivkin 1989), was enhanced by blue light. Our current result was inconsistent with these reports, in that the highest R_d was obtained under RL, with the value of 1.68 μmol O$_2$ g^{-1} h^{-1}. The R_d calculated in this study includes dark respiration and

photorespiration. In view of the former, one is not directly related to light, and it is highly likely that the photorespiration enhanced by RL affects the compensation point of alga, as presumed by Zhang et al. (1997). A higher R_d confirms a higher rate of carbohydrate degradation in RL; consequently, the growth rate is depressed. The compensation irradiance E_c was computed from the ratio of R_d to α. The highest light compensation point was found for RL, and the lowest was found for BL. The changes of E_c under different light qualities could be primarily attributed to changes in the R_d.

Consistent with the previous argument, the light intensity adopted in this study was far lower than the E_k of the plant, indicating that no photoinhibition occurred. Interestingly, significant changes in the chlorophyll *a* fluorescence parameter were observed under low light intensity (Fig. 7). The fluorescence F_m reflects the total amount of light absorbed by all the pigments in the algal cells (Matorin et al. 2004). The highest and lowest values of F_m were recorded under RL and BL conditions, respectively, which is consistent with the pigment contents, as shown in Fig. 3c. The higher values of F_v/F_m, qP, and Φ_{PSII} under BL suggested that both the photosynthetic activity and the electron transfer efficiency were improved by blue light. In contrast, RL had a significant negative effect on photosynthesis in *Dunaliella* sp. This negative effect of monochromatic red light has also been reported in higher plants, e.g., cucumber (Wang et al. 2009; Savvides et al. 2012), *Cordyline australis*, *Ficus benjamina*, and *Sinningia speciosa* (Zheng and Van Labeke 2017), despite the fact that red light coincides with the absorbance peak of chlorophyll and is known for its higher relative quantum efficiency than blue light in the instantaneous photosynthetic response (Mccree 1971). Generally, the excess light energy is quenched by photochemical reactions, nonphotochemical reactions, and

thermal dissipation. The nonphotochemical quenching of excitation energy, which protects higher plant photosynthetic machinery from photo damage, is triggered by acidification of the thylakoid lumen as a result of light-induced proton pumping, which also drives the synthesis of adenosine triphosphate (Goss and Lepetit 2015). In this study, in addition to the observation of the changes in the photochemical reaction under light-limiting treatment, we also found a significant change in NPQ. The higher total carotenoid content of microalga recorded under RL when compared to BL and WL was in accordance with many previous studies (Xu and Harvey 2019a, b; Han et al. 2019). It also be reported that the zeaxanthin content of alga exposed to RL was higher than that of BL (Xu and Harvey 2019b). The NPQ mechanism is controlled by the trans-thylakoid membrane pH gradient and the special xanthophyll cycle pigments, i.e., zeaxanthin, antheraxanthin, and violaxanthin (Gilmore 1997; Goss and Jakob 2010). The increase of NPQ under red light is probably caused by the enhancement of the xanthophyll cycle components' pool size. The actual mechanism behind this observation is beyond the scope of this study and would need to be investigated in future studies.

Recently, some studies tried to combine different LEDs with different absorption wavelengths to enhance the growth of plants, since LEDs with a wide variety of emission wavelengths are now available (Fu et al. 2013; Wagner et al. 2016; Hu and Sato 2017; Bian et al. 2018). In the batch cultivation of this study, the results clearly reflected that monochromatic RL increased the pigment contents. Moreover, BL was advantageous to the growth of *Dunaliella* sp. These results encouraged us to explore the growth response of *Dunaliella* sp. when these two monochromatic lights were provided simultaneously. The highest growth rate was achieved under dichromatic illumination (RL + BL) in a laboratory-scale PBR (Fig. 4), which could be attributed to increases in the light absorption efficiency or PSII efficiency in BL and pigment contents in RL. It was also reported that green light had positive effects on increasing the productivity of higher plants (Johkan et al. 2012; Wang et al. 2013; Bian et al. 2018). The growth rate under WL was far lower than that of RL + BL. We conclude that the green spectrum in WL has a negative effect on algal growth compared with monochromatic BL or RL, although more experiments are needed to examine this negative impact.

The cultivation employing a two-stage technology is more productive than batch and semicontinuous cultures, both in the tubular reactor and in the open tank, confirming that the two-stage cultures is the best approach for carotenoid production by *Dunaliella* (Prieto et al. 2011). The cell density of alga initially cultivated in stage one was 0.8×10^6 cells mL^{-1} (Ben-Amotz 1995). In a two-stage culture carried out by Tafreshi and Shariati (2006) under the climatic conditions of Iran in open ponds, the highest density of algal cells in the first stage was about 4.0×10^6 cells mL^{-1}. Meanwhile, the number of algal cells obtained in the reactor was 10 and 50 times of that in open pond obtained by Tafreshi and Shariati (2006) and Ben-Amotz (1995), respectively, with a biomass of 1.48 g L^{-1} (data not shown). This increase cannot be attributed solely to the differences in salinity, but to more steady manipulation of environmental factors, e.g., reduced light path, longer light duration, and continuous CO_2 supply (especially in the flat-plate PBR) (Borowitzka 1999; Tredici and Zitelli 1997). Our biomass result was lower than that of Prieto et al. (2011) obtained with the closed tubular PBR (2.2 g L^{-1}, also in F/2 medium), which may be attributed to the difference of light path. Many PBR types have been proposed (Tredici 2004), a proper efficiency and economy comparison still awaited for data from their simultaneous operation. It is gratifying to note that several recent studies have confirmed that red light can promote the accumulation of β-carotene in *D. salina*. For example, Han et al. (2019) proposed a lighting strategy named wavelength-shifting system (B-R system), that is, BL was first used to illuminate *D. salina* for 6 days (early stage), and then, the illuminating light was shifted to RL. Their results indicate that cells in the early stage showed enhanced density; however, *D. salina* under the B-R system showed enhanced density and β-carotene productivity compared to those under single LED light wavelengths (BL, WL, and RL). Xu and Harvey (2019a, b) found that red photon flux intensity controls the partitioning of electrons either for carotenoid biosynthesis or for photosynthesis, via energy absorption by chlorophyll and the plastoquinone pool, and further controls phytochrome regulation of the production of gene transcripts for phytoene synthase and β-carotene isomerases. In this study, the LR + LB illumination strategy was proposed for faster cell growth. It can be speculated that LR irradiation in the later period is a method worthy of further study to enhance the accumulation of carotenoids.

Based on the above results, we have drawn a model to summarize the findings of this study (Fig. 8). It should be
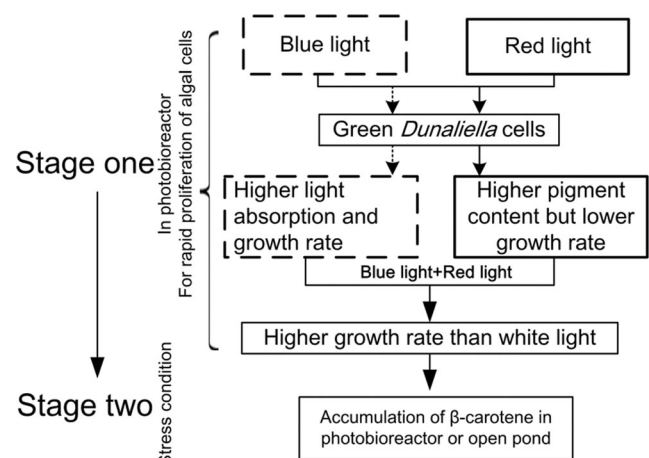

Fig. 8 The two-stage cultivation process of *D. salina* and our finding

noted that the results of this study are based on batch culture study and not as a semicontinuous or continuous one. Therefore, extrapolation of our results must be done with caution, and we acknowledge that studies on a chemostat cultivation are needed to verify our hypotheses, as adopted by Vadiveloo et al. (2015, 2016, 2017). Furthermore, the objective of this study was to identify the effect of light quality on the growth of *Dunaliella* sp. in a batch culture. The cultures that were maintained with various light spectra were not preacclimated to each light. However, our results can reflect the algal growth at different light qualities, which is still significant, whether light acclimation is completed or not (Rivkin 1989; Sánchez-Saavedra et al. 2016).

In conclusion, the photosynthesis of *Dunaliella* MACC/C43 was regulated by LED light quality. The mean extinction coefficient of this alga in the blue region was higher than those in the red, PAR, and green regions. Growth rate and photosynthesis both increased under BL, with enhanced F_v/F_m and qP values. Enhancement in the initial slope α and P_m and decreases in E_c and R_d, estimated from the P–E curve, were also observed. In contrast, RL-illuminated algae displayed a lower growth rate with a higher pigment content, compared with those under BL. Batch cultivation conducted in a PBR with different LED lights provided additional evidence that dichromatic illumination RL + BL was more suitable than monochromatic light, either RL or BL.

Compliance with ethical standards

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